

AWARD NUMBER: W81XWH-16-1-0576

TITLE: Deconstruction and Control of Neural Circuits in Posttraumatic Epilepsy

PRINCIPAL INVESTIGATOR: Dr. Jeanne Paz

CONTRACTING ORGANIZATION: J. David Gladstone Institutes
San Francisco, CA 94158

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2017		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2016 - 29 Sep 2017	
4. TITLE AND SUBTITLE Deconstruction and Control of Neural Circuits in Posttraumatic Epilepsy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-1-0576	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Jeanne Paz E-Mail: jeanne.paz@gladstone.ucsf.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The J. David Gladstone Institutes 1650 Owens St. San Francisco, CA 94158-2261				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT We pinpointed the hyperexcitable "hot spots" in the brain responsible for epileptic activities after TBI. These are located in the anterior portion of the injured neocortex in the S1 somatosensory cortex and in the functionally connected somatosensory portion of the thalamus. We found two anatomical and physiological biomarkers of the hot spots: 1) a massive upregulation of the C1q molecule, and 2) a reduced synaptic inhibition in these hot spots. These discoveries we made during the first funded year of the award pinpoint the neural circuits that we can now target to test two treatments in parallel. One treatment will consist in blocking the C1q effects by using the anti-C1q ANX005 drug; and the second treatment will consist in enhancing synaptic inhibition by human stem cell transplants in the "hot spots". We showed the feasibility of these two approaches and are starting to perform chronic recordings to determine the disease-modifying efficacy of the treatments. The results we will obtain during the next two years may lead to two treatments for preventing and/or treating the post-traumatic epilepsy after TBI. The long-term impact of this work will be to prevent, control, and cure post-traumatic epilepsy with no side effects, in contrast with the systemic treatment currently provided by anticonvulsants.					
15. SUBJECT TERMS Traumatic brain injury, Post-traumatic Epilepsy, Seizures, Neural Circuits, Inflammation, Gliosis, C1q molecule, Electrophysiology, Optogenetics, In vivo recordings during free behavior, Chronic EEG					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 20	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	1
3. Accomplishments.....	1
4. Impact.....	8
5.Changes/Problems.....	10
6. Products.....	11
7.Participants & Other Collaborating Organizations.....	13
8. Special Reporting Requirements.....	16
9. Appendices.....	16
10. Quad Chart.....	17

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

This proposal addresses significant gaps in the understanding of the pathophysiology of post-traumatic epilepsy. The **vision** of this study is to determine the causative links between TBI and epilepsy and prevent post-traumatic epilepsy. The **scope** of this study is to understand the mechanisms underlying the post-traumatic epilepsy, especially in service members and veterans. Despite decades of studies describing changes in the epileptic brain, we still lack clear answers to critical questions regarding precisely how a traumatic injury causes a non-epileptic brain to become epileptic. Dissecting causal links in a cell-type-specific manner became possible only recently due to the development of novel bioengineering tools by Dr. Deisseroth (previously Dr. Paz's postdoctoral mentor) and colleagues. These tools have not yet been applied to the study of post-traumatic epilepsy. We have applied optogenetics in a model of a different cortical insult—stroke—resulting in the first demonstration that targeting one specific cell type in a small brain region (within the thalamus) was sufficient to disrupt seizures. This work was awarded the Michael Prize 2015 for the best epilepsy research paper in the last two years. In this DoD-funded study we combine novel bioengineering tools with electrical engineering, signal processing, cellular and systems level neurophysiology, and a novel drug (ANX005, in collaboration with Annexon, Inc.) to dissect the causal links between TBI and PTE. During the first year of the award we pinpointed the “hot spots” in the brain that may be responsible for the development of PTE. This work laid ground to the next (ongoing) step of the study which will test two novel approaches to prevent and cure PTE: cutting edge human stem cell transplants to increase neural inhibition in specific brain regions and the Annexon drug to interfere with an inflammatory response to injury that is implicated in epileptogenesis. The **long-term goal** of this study is to develop novel treatments to prevent, control, and cure PTE with no side effects, in contrast with the systemic treatment currently provided by anticonvulsants.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Traumatic brain injury, Post-traumatic Epilepsy, Seizures, Neural Circuits, Inflammation, Gliosis, C1q molecule, Electrophysiology, Optogenetics, In vivo recordings during free behavior, Chronic EEG

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

There were no changes in the project or its direction.

- **What were the major goals of the project?**

- *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

Below we report the percentage of completion for the period relevant for this yearly report.

Major Task 1: Determine the role of corticothalamocortical hyperexcitability in seizures after TBI.	Months	Percentage of completion
Aim 1a: Determine if TBI results in hyperexcitability in CTC circuits	1-7	98%
Aim 1b: Determine if the thalamus can bi-directionally control PTE seizures in freely behaving animals.	1-7	80%
Milestone(s): unveil the epileptic circuit hot spots and specific cells that are causally involved in PTE.	7	
Major Task 2: Determine the role of C1q in seizures after TBI.		
Aim 2a: Determine if C1q is upregulated in CTC “hot spots” after TBI	1-7	98%
Aim 2b: Determine if blocking C1q action prevents PTE	1-12	50%
Aim 2c: Determine if blocking C1q cures PTE	8-17	5%
Milestone(s): Reveal the role of the immune response involving C1q in circuit plasticity after TBI, validate a new biomarker (C1q and thalamic gliosis) for the epileptogenesis in PTE, and determine if the drug ANX005 that blocks the effects of C1q is efficient in preventing and curing PTE.	17	
Major Task 3: Determine if transplanting inhibitory neurons into CTC “hot spots” of hyperexcitability prevents and cures PTE.		
Aim 3a: Determine if inhibitory transplants in cortex and thalamus prevent circuit hyperexcitability in cortical and thalamic slices, respectively	6-12	20%
Aim 3b: Determine if transplanted cells prevent PTE in behaving animals.	13-24	—
Aim 3c: Determine if transplanted cells cure PTE in behaving animals.	24-36	—
Milestone(s): Test the efficacy of a novel therapeutic approach involving mouse and human cell transplants for preventing PTE and cure PTE.	36	—

- **What was accomplished under these goals?**
 - *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the*

emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) major activities;

Major activities during the first year of the award:

-All the protocols necessary to carry on the proposed projects were approved including IACUC and BUA.

-We purchased a cortical impactor (Leica) and optimized the cortical impact approach in mice to ensure reproducibility of the location and size of the cortical lesions.

-We purchased a TDT recording system that allows to record the electrophysiological activities after TBI *in vitro* and *in vivo*.

-We performed the experiments that aim to locate the “network hot spots” after TBI during the latent phase before the chronic epilepsy develops (i.e. within the first 30 days post-TBI). Briefly, we performed extracellular multi-unit and local field potential recordings in brain slices from mice with cortical TBI and sham mice with no injury. A 16-channel recording probe (Neuronexus) was placed in either the somatosensory thalamus or the somatosensory cortex ipsilateral to the TBI, and contralateral to the TBI. We stimulated was delivered to the neighboring white matter tract to evoke firing or field responses in the thalamus and cortex.

-We performed immunohistochemical studies to identify the extent and duration of inflammatory responses after injury. Mice with TBI were perfused at different time points after injury, and brains were sectioned and stained for markers of inflammation (GFAP, Iba1, C1q) and of neurons (NeuN) to determine location and extent of the neuronal loss.

-We purchased and finished setting up new equipment (DSI company) for chronic EEG recordings during free behavior. Setting up this new equipment was a major milestone given that it is required to achieve our goals outlined in Aim 2b and Aim 3.

-We finished setting up a new TDT recording system and started recording EEG and multiunit depth activities in the thalamus (Aim 1b). This setup allowed us to start experiments that aim to assess the electrophysiological activities after TBI, and perform optogenetic manipulations to disrupt seizures.

These optogenetic experiments were delayed because of an IACUC error that expired the protocol which delayed the experiments by a month. The issue is now resolved, the protocol is renewed by IACUC and we expect to finish the optogenetic experiments depicted in Aim 1b during the next quarterly reporting period.

-We continued immunohistochemical studies started in the first quarterly period to identify the extent and duration of inflammatory responses after injury. Mice with TBI were perfused at different time points after injury, and brains were sectioned and stained for markers of inflammation (GFAP, Iba1, C1q) and of neurons (NeuN) to determine location and extent of the neuronal loss. We are currently quantifying the immunohistochemical results, including the location and the amount of GFAP, Iba1 and C1q in thalamus and peri-injured cortex.

-We started quantifying the effect of the anti-C1q drug on the expression levels of C1q molecule and the downstream C3 molecule in thalamus and cortex of sham animals and on the circuit hyperexcitability in the “hot spots”. The results are being currently analyzed.

-We had a meeting with the company Annexon Inc on July 26th to obtain the ANX005 anti-C1q antibody for *in vivo* studies.

-We are finalizing the in depth analysis of the electrophysiological recordings from slices from mice treated or not with the anti-C1q antibody.

-We met with collaborators (Drs Nicholas and Kriegstein, *Neurona Inc*) who provided the human cells for transplants for Aim 3. We showed that the human cells survive in the mouse brain despite the inflammation due to the brain lesion.

2) *specific objectives;*

The objectives of the experiments performed during this funding period are listed below:

1. Determine if TBI results in hyperexcitability in CTC circuits and locate the “hot spots” (Aim 1a)

This objective was accomplished. The data analysis is being completed.

2. Determine if optogenetic targeting of the thalamus can initiate or suppress seizures (Aim 1b)

The animals are ready to be recorded. This aim was slightly delayed because of a clerical issue at IACUC and a sudden unexpected expiration of our protocol. The issue is now resolved and these experiments will be finalized during the next quarterly period.

3. Determine if C1q is upregulated in CTC “hot spots” after TBI (Aim 2a)

This objective was accomplished. The data analysis is being completed.

4. Determine if blocking C1q action prevents circuit hyperexcitability identified in the aims outlined above (Aim 2)

This objective was accomplished. The data analysis is being completed.

3) *significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative);*

Significant results:

1. We were able to locate the circuit “hot spots” in the cortex and thalamus in TBI mice. We completed the analysis of the experiments that were performed during the previous funding period and that aimed to identify the “hot spots” within the CTC circuits within the latent period (<4 weeks post-TBI, i.e. before epilepsy develops). Our results, obtained 3-4 weeks post-TBI, show that the evoked cortical network activity is altered in the superficial neocortical layers (Layers 1-4). We showed that the circuit excitability

is enhanced only in the peri-lesion cortex but not in the corresponding cortex that is contra-lateral to the injury. Our results suggest that the peri-lesion cortex is hyperexcitable before the development of PTE. We are currently quantifying the location and extent of the inflammation and its timeline with the Image J software.

2. We found persistent upregulation of C1q in the peri-lesion cortex and the functionally connected thalamus 3-4 weeks post-TBI. Notably, we found that the C1q upregulation is co-localized with changes in cellular and circuit hyperexcitability. These results suggest that C1q upregulation is a biomarker for circuit abnormalities after TBI, during the epileptogenic phase (i.e. before PTE develops). Interestingly, we found that 3-4 weeks after TBI the peri-lesion cortex shows a stronger circuit hyperexcitability than the thalamus. This result suggests that during the epileptogenic phase (i.e. before PTE develops) the cortex is more hyperexcitable than the thalamus.

3. We showed the ANX005 (anti-C1q drug) penetrates in the brain and reduces chronic inflammation.

4. We showed the human cells survive in the inflamed mouse brain tissue.

5. We found potential EEG biomarkers for PTE.

6. We showed the thalamic hot spots are involved in the early seizure expression after TBI (during the latent phase). Optrode recordings in the thalamus showed high frequency burst of action potential firing during non convulsive seizure “silent” events.

7. We showed that TBI lowers the PTZ-induced seizure threshold during the latent phase, i.e. before epilepsy develops.

8. We are now starting chronic EEG recordings that will allow us to test two therapeutic strategies to prevent PTE: ANX005 (anti-C1q drug) treatments and human stem cell transplants.

4) other achievements.

-This study resulted in multiple awards to the students who work on this project in my lab. Two graduate students in my laboratory - Stephanie Holden and Frances Cho –received awards that allowed them to present their work at multiple national and international conferences. These awards include the ARCS fellowship for two years in a row (2016 and 2017), the Doctoral Career development Award from the *Society for Neuroscience* as well as travel awards from the UCSF graduate division to attend conferences and present the work on the mechanisms underlying post-traumatic epileptogenesis.

-The results of the study funded by this award were presented this work at multiple conferences:

1. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
2. Cho F, Paz JT (2017) “Assessing the effect of astrogliosis on thalamic circuit excitability”. Young Generation Technical and Leadership Conference 2017 (YGTLC). Hosted by the Korean-American Scientist and Engineers Association, Korea (selected for a talk).

3. Holden S, Paz JT (2016) "The Role of the thalamus in focal traumatic brain injury". ARCS Symposium 2016, Menlo Park, CA, USA.
4. Holden S, Paz JT (2016) "Thalamic excitability after traumatic cortical injury". Gordon research conference -Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain.
5. Cho F, Makinson S, Holden S, Tager D, Paz JT (2016) "Selective astrogliosis increases thalamic circuit excitability and oscillations". GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain ([abstract selected for a talk](#)).
6. Holden S, Paz JT (2016) "Thalamic function and traumatic cortical injury". Gordon research conference on Thalamus. Ventura, CA, USA.
7. Cho F, Makinson S, Holden S, Paz JT (2016) Assessing the effect of astrogliosis n thalamic circuit excitability". Gordon research conference on Thalamus. Ventura, CA, USA.

- **What opportunities for training and professional development has the project provided?**
 - *If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*
 - *Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

I require each of the trainees in my lab to complete a written Individual Development Plan (IDP) each year, and we meet formally at the end of the year to discuss their overall development. In addition, I meet with each trainee weekly to discuss progress during the week. For the first 2 years of a graduate student's tenure in my lab, I advise them to use "My Annual Plan" (MAP), which was created by UCSF's Graduate Division and focuses the student's efforts on broad training goals and accomplishments. Postdoctoral fellows participate in Gladstone's annual performance goal setting and appraisal program, which includes a thorough discussion of training milestones and advancement. Both graduate students and postdoctoral fellows are encouraged to use Science Careers' *myIDP* which provides more structure for considering a broad array of anticipated career outcomes and encourages productive goal setting toward the pursuit of a chosen career. Postdocs are also advised to use *myIDP* throughout their tenure at Gladstone.

During the funding period, I mentored four minority trainees in my laboratory who were involved in post-injury epilepsy studies: a graduate student (Alexandra Clemente-Perez), a research associate (Juan Alcauter), and two summer interns – Charell Sherman and Trinidad Arceo – from the *Promoting Underrepresented Minorities Advancing in the Sciences (PUMAS)* internship program.

This award also provided a training opportunity for an undergraduate SRTP student (Summer Research Training Program, Office of Outreach and Diversity, UCSF). SRTP students are fully funded undergraduates with previous research experience who show exceptional promise as research scientists. They attend mentoring sessions throughout the summer, but are otherwise full-time in the lab, and are obligated to present their work in a poster session at the end of the summer. My SRTP mentee, Allison Morningstar, received training during his project and was involved in immunohistology and data analysis.

Two graduate students in my laboratory - Stephanie Holden and Frances Cho – whose work focuses on this DoD-funded project, received multiple awards that allowed them to present their work at multiple national and international conferences that gather the experts in the field which created important training and networking opportunities for the students. These awards include the ARCS fellowship for two years in a row (2016 and 2017), the Doctoral Career development Award from the Society for Neuroscience as well as UCSF travel awards to attend conferences and present their work on the mechanisms underlying post-traumatic epileptogenesis. Stephanie and Frances presented their work at multiple conferences:

8. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
9. Cho F, Paz JT (2017) “Assessing the effect of astrogliosis on thalamic circuit excitability”. Young Generation Technical and Leadership Conference 2017 (YGTLC). Hosted by the Korean-American Scientist and Engineers Association, Korea (selected for a talk).
10. Holden S, Paz JT (2016) “The Role of the thalamus in focal traumatic brain injury”. ARCS Symposium 2016, Menlo Park, CA, USA.
11. Holden S, Paz JT (2016) “Thalamic excitability after traumatic cortical injury”. Gordon research conference -Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain.
12. Cho F, Makinson S, Holden S, Tager D, Paz JT (2016) “Selective astrogliosis increases thalamic circuit excitability and oscillations”. GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain (abstract selected for a talk).
13. Holden S, Paz JT (2016) “Thalamic function and traumatic cortical injury”. Gordon research conference on Thalamus. Ventura, CA, USA.
14. Cho F, Makinson S, Holden S, Paz JT (2016) Assessing the effect of astrogliosis n thalamic circuit excitability”. Gordon research conference on Thalamus. Ventura, CA, USA.

▪ **How were the results disseminated to communities of interest?**

- *If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

The results obtained during the first year of the funding period were disseminated to communities of interest through presentations at conferences:

1. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
2. Cho F, Paz JT (2017) “Assessing the effect of astrogliosis on thalamic circuit excitability”. Young Generation Technical and Leadership Conference 2017 (YGTLC). Hosted by the Korean-American Scientist and Engineers Association, Korea (selected for a talk).
3. Holden S, Paz JT (2016) “The Role of the thalamus in focal traumatic brain injury”. ARCS Symposium 2016, Menlo Park, CA, USA.

4. Holden S, Paz JT (2016) "Thalamic excitability after traumatic cortical injury". Gordon research conference -Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain.
5. Cho F, Makinson S, Holden S, Tager D, Paz JT (2016) "Selective astrogliosis increases thalamic circuit excitability and oscillations". GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain (abstract selected for a talk).
6. Holden S, Paz JT (2016) "Thalamic function and traumatic cortical injury". Gordon research conference on Thalamus. Ventura, CA, USA.
7. Cho F, Makinson S, Holden S, Paz JT (2016) Assessing the effect of astrogliosis on thalamic circuit excitability". Gordon research conference on Thalamus. Ventura, CA, USA.

▪ **What do you plan to do during the next reporting period to accomplish the goals?**

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan 1) to complete the work proposed in Aim 1 and finish the analysis of the results, 2) to continue the work proposed in Aim 2, and 3) to start working on Aim 3. Briefly, this year our work pinpointed the "hot spots" of neural dysfunction after TBI. These are located in the peri-injured cortex and the functionally connected somatosensory thalamus. We used histological and electrophysiological approaches in brain slices and in vivo to locate the "hot spots" of circuit dysfunction and characterize their evolution in time and space. Our results suggest that the chronic upregulation in C1q and the reduced of synaptic inhibition are the biomarkers of the "hot spots". We also purchased and set up a chronic EcoG recording setup which will allow us to monitor the ECoG for multiple months after TBI. Furthermore, we validated the penetration of the ANX005 anti-C1q drug in the brain regions of interest, and we found the optimal conditions for the treatment (dose and frequency of administration). Thanks to the progress we made during year 1, we are starting the experiments that will allow us to assess the efficacy of two novel treatments for the post-traumatic epileptogenesis: 1) anti-C1q drug (ANX005) treatment (Aim 2), and 2) stem cell treatment (Aim 3). We hypothesize that these two treatments will prevent post-traumatic epileptogenesis by reducing inflammation and restoring the inhibition/excitation balance.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

▪ **What was the impact on the development of the principal discipline(s) of the project?**

- *If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

This proposal addresses significant gaps in the understanding of the pathophysiology of post-traumatic epilepsy, which are central to the ERP's vision and mission.

Short-Term Impact: Tangible intellectual gains from these studies will include novel therapeutic targets for post-traumatic epileptogenesis (PTE) and effective PTE seizure control (regions, cells,

and circuits) that have been thoroughly characterized in terms of their *in vitro* and *in vivo* physiological relevance. Our study identified the hyperexcitable “hot spots” using neuroanatomical and high-throughput electrophysiological assays (*Aim 1*). We start to target these regions in a translational context relevant for preclinical studies with the drug ANX005 (*Aim 2*), and human interneuron cell transplants (*Aim 3*) to reverse epilepsy after TBI or prevent PTE from developing after injury.

Long-Term Impact: Our ongoing study aims to develop novel treatments to prevent, control, and cure PTE with no side effects, in contrast with the systemic treatment currently provided by anticonvulsants. We expect that a better understanding of the underlying pathophysiological mechanisms of PTE will lead to development of novel treatments that target relevant brain circuits identified in our anatomical screen. Thus, this proposal will provide insight into long-term strategies that might represent a more focal therapeutic intervention for people suffering from epilepsy. The current gold standard for epilepsy treatment, regardless of its origin, is anticonvulsant drugs. These drugs are ineffective in 30% of patients with epilepsy and cause a variety of negative side effects. Short-term side effects include tremors, sedation, and loss of coordination. Long-term effects include a higher incidence of birth defects, the generation of liver and heart disease, and additional seizures and other neuropsychiatric symptoms, such as psychosis (Center for Epilepsy and Seizure Education). The novel preventive (ANX005 drug in *Aim 2*) or focal treatments (human cell transplants in *Aim 3*) would diminish the activation of off-target tissues, possibly reducing negative side effects.

▪ **What was the impact on other disciplines?**

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Our study has a fundamental interest given that it will reveal the role of C1q complement pathway in neural circuit plasticity. We find a pathological upregulation of C1q in the thalamus of a rat model of cortical thrombotic stroke, suggesting that C1q upregulation could represent a biomarker for chronic circuit abnormalities that lead to the development of epilepsy.

▪ **What was the impact on technology transfer?**

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including: transfer of results to entities in government or industry; instances where the research has led to the initiation of a start-up company; or adoption of new practices.*

N/A

▪ **What was the impact on society beyond science and technology?**

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*
 - *improving public knowledge, attitudes, skills, and abilities;*
 - *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
 - *improving social, economic, civic, or environmental conditions.*

This study could lead to disease modifying treatments for post-traumatic epilepsy that will enhance quality of life and reduce the social and economic burden associated with deadly intractable seizures and associated cognitive deficits.

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Changes in approach and reasons for change

- *Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Nothing to Report.

▪ **Actual or anticipated problems or delays and actions or plans to resolve them**

- *Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Nothing to Report.

▪ **Changes that had a significant impact on expenditures**

- *Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Nothing to Report.

▪ **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

- *Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

We recently discovered that an error in the IACUC office in February 2015 gave a three-year expiration on a protocol that was approved months earlier with a June 2017, 3-year expiration. That has now been resolved and IACUC has approved the February 2015 review as a de novo protocol that re-started the 3-year approval cycle. Once the error was discovered we stopped all animal work until it was resolved. This delayed progress on aims 1b, 2b and 3a. This delay was minor and will not affect the progress of the other aims and we intend to be on track for the next quarterly report.

Aim 1b: Determine if the thalamus can bi-directionally control PTE seizures in freely behaving animals.

We were supposed to be accomplished this Aim by now. The issues with IACUC delayed the completion of this experiment so we accomplished only 80% of this Aim. The animals are ready but we will need to collect data to determine whether the thalamic cells are sufficient to bi-directionally modulate seizures after TBI. We expect to finish these experiments during the next quarterly period.

Aim 2b: Determine if blocking C1q action prevents PTE.

We were supposed to be accomplished this Aim by now. The issues with IACUC delayed the completion of this experiment so we accomplished only 50% of this Aim. The animals are ready but we will need to collect data to determine whether blocking the effects of the C1q molecule with ANX005 treatment can prevent PTE. We expect to finish these experiments during the next quarterly period.

Aim 3a: Determine if inhibitory transplants in cortex and thalamus prevent circuit hyperexcitability in cortical and thalamic slices, respectively.

We were supposed to be accomplished this Aim by now. The issues with IACUC delayed the completion of this experiment so we accomplished only 20% of this Aim. We showed the feasibility of this approach by demonstrating that the human stem cells survive in the mouse brain despite the inflammation after TBI. We expect to finish these experiments during the next quarterly period.

Significant changes in use or care of human subjects

N/A

- **Significant changes in use or care of vertebrate animals.**
- N/A
- **Significant changes in use of biohazards and/or select agents**
- N/A

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

- **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*
- N/A
- **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Cho F, Clemente A, Holden S, Paz JT (2017) Thalamic Models of Seizures In Vitro. In: Models of Seizures and Epilepsy, 2nd edition. Eds. Pitkänen A, Buckmaster P, Galanopoulou AS, Moshe SM. Chapter 19.

- **Other publications, conference papers, and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

The results obtained during the first year of the award were presented at multiple national and international conferences:

1. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
2. Cho F, Paz JT (2017) “Assessing the effect of astrogliosis on thalamic circuit excitability”. Young Generation Technical and Leadership Conference 2017 (YGTLC). Hosted by the Korean-American Scientist and Engineers Association, Korea (selected for a talk).
3. Holden S, Paz JT (2016) “The Role of the thalamus in focal traumatic brain injury”. ARCS Symposium 2016, Menlo Park, CA, USA.
4. Holden S, Paz JT (2016) “Thalamic excitability after traumatic cortical injury”. Gordon research conference -Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain.
5. Cho F, Makinson S, Holden S, Tager D, Paz JT (2016) “Selective astrogliosis increases thalamic circuit excitability and oscillations”. GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain (abstract selected for a talk).
6. Holden S, Paz JT (2016) “Thalamic function and traumatic cortical injury”. Gordon research conference on Thalamus. Ventura, CA, USA.
7. Cho F, Makinson S, Holden S, Paz JT (2016) Assessing the effect of astrogliosis on thalamic circuit excitability”. Gordon research conference on Thalamus. Ventura, CA, USA.

a. **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

N/A

b. **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

▪ N/A

c. **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

▪ N/A

d. **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life.

▪ N/A

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

d. **What individuals have worked on the project?**

- i. *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

Example:

Name:	Dr. Jeanne Paz
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	1234567

Nearest person month worked:	5
Contribution to Project:	<i>Ms. Smith has performed work in the area of combined error-control and constrained coding.</i>
Funding Support:	<i>The Ford Foundation (Complete only if the funding support is provided from other than this award).</i>

1. Name: Jeanne Paz

Project Role: PI

Nearest person month worked: 2

Contribution to Project: provided technical and conceptual guidance on all aspects of the project: cortical lesions / Optrode and EEG device design and surgical implants, data collection / data analysis / data interpretation / project management.

2. Name: Scott Brovarney

Project Role: Research Associate

Nearest person month worked: 2

Contributions to project: Mr. Brovarney has aided in completing documentation to sustain all the protocol approvals required for this project, was responsible for managing lab supplies, equipment and mouse colonies, helped with immunohistology, and performed experiments related to the inflammatory response and C1q upregulation after brain lesions.

3. Name: Stefanie Makinson

Project Role: Graduate Student

Nearest person month worked: 1

Contribution to Project: Ms. Makinson has done all of the TBI surgeries, carried out the whole-cell patch-clamp and extracellular multi-unit recordings in brain slices, performed histology on brain sections from mice with TBI, made Optrode and EEG devices and performed surgical implants and electrophysiological (EEG and multi-unit) recordings in freely behaving mice, as well as chronic EEG recordings.

4. Juan Alcauter

Project Role: Graduate Student

Nearest person month worked: 1

Contribution to Project: Mr. Alcauter has carried out the local field potential recordings in acute neocortical brain slices from mice with TBI and the littermate controls, and was involved in analyzing the EEG from chronically implanted TBI and sham mice.

5. Bryan Higashikubo

Project Role: Postdoctoral fellow

Nearest person month worked: 1

Contribution to Project: Bryan was actively involved in purchasing and setting up the equipment for chronic EEG recordings from freely behaving mice; in surgical implants of chronic EEG devices in mice with TBI, in data acquisition and analysis. Bryan's programming skills from his PhD training at MIT are an important asset for managing and analyzing the large data sets that we obtain from chronic recordings.

e. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- i. *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- ii. *If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

-Scott Brovarney was promoted to a different job and his work related to the current project is now performed by Juan Alcauter (2017 BS graduate from UC Berkeley, Research Associate I and Lab manager in Paz lab since July 2017).

-Stefanie Makinson obtained a job as a group leader in industry and left the lab. Her slice related work of the project is now performed by Frances Cho.

-Bryan Higashikubo (2016 PhD MIT) joined the Paz lab as a postdoctoral fellow and will actively work on the chronic EEG recordings and analysis that are necessary in Aims 2 and 3. Bryan's programming skills from his PhD training at MIT are an important asset for managing and analyzing the large data sets that we obtain from chronic recordings.

f. What other organizations were involved as partners?

- i. *If there is nothing significant to report during this reporting period, state "Nothing to Report."*

Nothing to Report.

- ii. *Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

Nothing to Report.

Provide the following information for each partnership:

1. **Organization Name:**
2. **Location of Organization:** *(if foreign location list country)*
3. **Partner's contribution to the project** *(identify one or more)*
 - a. **Financial support;**

- b. **In-kind support** (*e.g., partner makes software, computers, equipment, etc., available to project staff*);
- c. **Facilities** (*e.g., project staff use the partner's facilities for project activities*);
- d. **Collaboration** (*e.g., partner's staff work with project staff on the project*);
- e. **Personnel exchanges** (*e.g., project staff and/or partner's staff use each other's facilities, work at each other's site*); and
- f. **Other.**

8. **SPECIAL REPORTING REQUIREMENTS**

- a. **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*
 - b. **QUAD CHARTS:** *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*
9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***

Insert Project Title Here

Log #EP150038 and Deconstruction and Control of Neural Circuits in Epilepsy
W81XWH-16-1-0576



PI: Dr. Jeanne Paz

Org: The J. David Gladstone Institutes

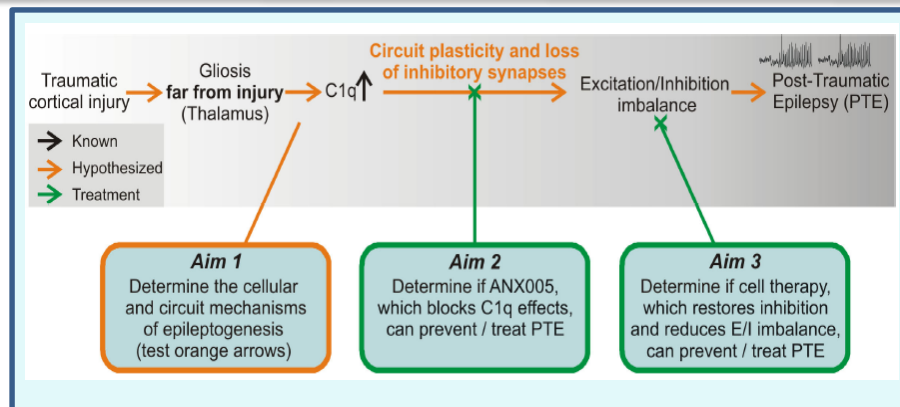
Award Amount: \$875,289

Study/Product Aim(s)

- Aim 1: Determine the role of corticothalamocortical hyperexcitability in seizures after TBI.
- Aim2: Determine the role of C1q in seizures after TBI.
- Aim 3: Determine if transplanting inhibitory neurons into CTC “hot spots” of hyperexcitability prevents and cures PTE.

Approach

To achieve these goals we use a mouse model in which post-traumatic epilepsy develops after a traumatic cortical injury induced by a controlled cortical impact method. To understand how epilepsy develops after TBI and to test two new disease-modifying treatments, we combine cellular and circuit electrophysiology *in vitro* and in freely behaving mice, optogenetic approaches, pharmacology, and chronic electrocorticogram (EcoG) recordings.



Accomplishment: We located the neural “hot spots” responsible for the generation of epileptic activities after TBI. We characterized these hot spots and found their “biomarkers”: local gliotic inflammation, upregulation of the C1q molecule and a reduced synaptic inhibition. Using chronic EcoG recordings, we found biomarkers of post-traumatic epileptogenesis.

Timeline and Cost

Activities	CY	15	16	17	18
Text (Major aim/study/milestone)					
Text (Major aim/study/milestone)					
Text (Major aim/study/milestone)					
Text (Major aim/study/milestone)					
Estimated Budget (\$K)		\$000	\$000	\$000	\$000

Goals/Milestones (Example)

CY15 Goal – System demonstration

☒ Functionality tests of integrated firmware and software

CY16 Goals – System validation

☐ Investigate earplug designs based on collected features

☐ Complete formal attenuation and comfort trials of earplugs

CY17 Goal – Production readiness

☐ Validate design architecture for digital ear canal volumetric shape capture and data workflow

CY18 Goal – Navy suitability testing

☐ Field test on aircraft carrier flight deck

Comments/Challenges/Issues/Concerns

• If timelines change, comment here.

• If off by more than one quarter in spending, comment here.

Budget Expenditure to Date

Projected Expenditure:

Actual Expenditure:

Updated: Oct 30th 2017